

WE CLAIM:

1 1. A method for producing and identifying an active doublestranded
2 RNA (dsRNA) which attenuates a desired gene expression in a cell, said method
3 comprising:

- 4 (a) producing a plurality of cDNA, wherein each cDNA comprises at
5 least a portion of a gene that is expressed in a cell;
6 (b) producing a candidate dsRNA from at least one of the cDNA;
7 (c) introducing the candidate dsRNA into a reference cell; and
8 (d) identifying an active dsRNA by determining whether the candidate
9 dsRNA modulates a desired candidate gene expression in the reference cell.

1 2. The method of Claim 1 further comprising producing the identified
2 active dsRNA from a corresponding cDNA of step (a).

1 3. The method of Claim 1, wherein said step of identifying the active
2 dsRNA comprises:

3 (A) selecting a candidate gene, wherein the candidate gene is a gene
4 that is expressed in a test cell and/or a control cell, and/or is expressed at a detectably
5 different level with respect to the test cell and the control cell, and the test cell and control
6 cell differ with respect to a cellular characteristic; and

7 (B) identifying whether the candidate dsRNA is an active dsRNA by
8 determining whether down-regulation of expression of the candidate gene in a reference
9 cell has a functional effect in the reference cell, wherein the determining step comprises:

10 (i) introducing the candidate dsRNA which is substantially
11 identical to at least a part of the candidate gene into the
12 reference cell; and

13 (ii) detecting an alteration in a cellular activity or a cellular
14 state in the reference cell, alteration indicating that the
15 candidate gene plays a functional role in the reference cell
16 and is an active dsRNA.

1 4. The method of Claim 1, wherein said step of producing a plurality
2 of cDNA comprises:

- 3 (i) isolating at least one mRNA from the cell, and

4 (ii) producing a double-stranded cDNA from the isolated mRNA by
5 reverse transcription.

1 5. The method of Claim 4, wherein step of producing a plurality of
2 cDNA further comprises producing cDNAs of a similar length by digesting cDNA of said
3 step (ii) with a restriction enzyme.

1 6. The method of Claim 5, wherein said step (b) of producing the
2 candidate dsRNA comprises:

3 (i) producing a plasmid or PCR fragment from the cDNA, and
4 (ii) producing the candidate dsRNA from the plasmid or PCR
5 fragment.

1 7. The method of Claim 6, wherein the plurality of cDNA comprises
2 at least a portion of substantially all genes that are actively expressed in the cell.

1 8. The method of Claim 6, wherein the desired affect of the candidate
2 dsRNA on the reference cell is a result of the candidate dsRNA attenuating expression of
3 a candidate gene in the reference cell.

1 9. The method of Claim 8, wherein the candidate dsRNA has
2 complete sequence identity with the candidate gene over at least 100 nucleotides.

1 10. The method of Claim 9, wherein the candidate dsRNA has
2 complete sequence identity with the candidate gene over at least 500 nucleotides.

1 11. The method of Claim 1, wherein the candidate dsRNA is at least
2 100 nucleotides in length.

1 12. The method of Claim 11, wherein the candidate dsRNA is at least
2 500 nucleotides in length.

1 13. The method of Claim 12, wherein the candidate dsRNA is between
2 500 and 1100 nucleotides in length.

1 14. A method for identifying and validating the effect of an active
2 double-stranded RNA (dsRNA) which attenuates a desired gene expression in a cell, said
3 method comprising:

4 (a) producing a candidate dsRNA which comprises at least a portion of
5 a candidate gene that is expressed in a control cell;
6 (b) introducing the candidate dsRNA into a reference cell; and
7 (c) identifying whether the candidate dsRNA is an active dsRNA by
8 detecting an alteration in a cellular activity or a cellular state in the reference cell,
9 alteration indicating that the candidate gene plays a functional role in the reference cell
10 and is an active dsRNA.

1 15. The method of Claim 14, wherein said step of producing the
2 candidate dsRNA comprises:

3 (i) producing a cDNA from a mRNA of the control cell such that the
4 cDNA comprises at least a portion of the gene that is expressed in the control cell; and
5 (ii) producing the candidate dsRNA from at least one of the cDNA of
6 said step (i).

1 16. The method of Claim 14, wherein the candidate gene is a gene that
2 is expressed in a test cell and/or the control cell, and/or is expressed at a detectably
3 different level with respect to the test cell and the control cell, and the test cell and control
4 cell differ with respect to a cellular characteristic.

1 17. A method for correlating genes and gene function, said method
2 comprising:

3 (a) producing a plurality of candidate dsRNAs from a plurality of
4 cDNAs of a control cell such that each candidate dsRNA comprises at least a portion of a
5 gene that is expressed in the control cell;

6 (b) introducing each of the candidate dsRNA into a plurality of
7 separate reference cell each having a gene expression similar to the control cell in step
8 (a); and

9 (c) identifying which candidate dsRNA is an active dsRNA by
10 detecting an alteration in a cellular activity or a cellular state in the reference cell, desired
11 alteration indicating that the gene corresponding to the candidate dsRNA plays a
12 functional role in the reference cell.

1 18. The method of Claim 17, wherein the plurality of cDNAs is
2 produced from a plurality of mRNAs which are produced by the control cell.

1 19. The method of Claim 18, wherein said step of producing a plurality
2 of cDNA comprises:

- 3 (i) isolating at least one mRNA from the cell;
4 (ii) producing a double-stranded cDNA from the isolated mRNA by
5 reverse transcription;
6 (iii) producing cDNAs of a similar length by digesting cDNA of said
7 step (ii) with a restriction enzyme; and
8 (iv) producing a plasmid or PCR fragment from the cDNA of said step
9 (iii).

1 20. The method of Claim 19, wherein the candidate dsRNA is
2 produced by transcribing the plasmid cDNA or PCR fragment of said step (iv).

1 21. The method of Claim 19, wherein the plurality of cDNA comprises
2 at least a portion of substantially all genes that are actively expressed in the cell.

1 22. The method of Claim 19, wherein the restriction enzyme is selected
2 from the group consisting of Dpn1 and Rsa1.

1 23. The method of Claim 17, wherein said step of producing the
2 plurality of candidate dsRNAs comprises:

3 (A) selecting a candidate gene, wherein the candidate gene is a gene
4 that is expressed in a test cell and/or a control cell, and/or is expressed at a detectably
5 different level with respect to the test cell and the control cell, and the test cell and control
6 cell differ with respect to a cellular characteristic; and

7 (B) producing the plurality of candidate dsRNAs, wherein each
8 candidate dsRNA is substantially identical to at least a part of the candidate gene.

1 24 The method of claim 23, wherein the candidate gene is selected
2 from a normalized library prepared from cells of the same type as the test cell or the
3 control cell and is present in low abundance in the normalized library.

1 25. The method of claim 23, wherein the candidate gene is a
2 differentially expressed gene selected from a subtracted library that is enriched for genes
3 that are differentially expressed with respect to the test cell and the control cell.

1 26. The method of claim 25, wherein the subtracted library is also
2 normalized and the candidate gene is one of the genes that is both present in low
3 abundance and differentially expressed in the subtracted and normalized library.

1 27. The method of claim 23, wherein said step of selecting the
2 candidate gene comprises:

3 (i) preparing

4 (A) a tester-normalized cDNA library which is a normalized library
5 prepared from test cells;

6 (B) a driver-normalized cDNA library which is a normalized library
7 prepared from control cells;

8 (C) a tester-subtracted cDNA library which is enriched in one or more
9 genes that are up-regulated with respect to the test cell and the
10 control cell, and

11 (D) a driver-subtracted cDNA library which is enriched in one or more
12 genes that are down-regulated with respect to the test cell and the
13 control cell; and

14 (ii) identifying one or more clones from the normalized libraries and/or the
15 subtracted libraries,

16 wherein the candidate gene is one of the clones identified.

1 28. The method of Claim 27, wherein said step of identifying one or
2 more clones from the normalized libraries comprises:

3 (A) contacting clones from the tester-normalized cDNA library with
4 labeled probes derived from mRNA from test cells and contacting clones from the driver-
5 normalized cDNA library with labeled probes derived from mRNA from control cells
6 under conditions whereby probes specifically hybridize with complementary clones to
7 form a first set of hybridization complexes; and

8 (B) detecting at least one hybridization complex from the first set of
9 hybridization complexes to identify a clone from one of the normalized libraries which is
10 present in low abundance.

1 29. The method of Claim 27, wherein said step of identifying one or
2 more clones from the subtracted libraries comprises:

3 (A) contacting clones from the tester-subtracted cDNA library and
4 contacting clones from the driver-subtracted cDNA library with a population of labeled
5 probes under conditions whereby probes from the population of probes specifically
6 hybridize with complementary clones to form a second set of hybridization complexes,
7 and wherein the population of labeled probes is derived from mRNA from test cells and
8 control cells; and

9 (B) detecting at least one hybridization complex from the second set of
10 hybridization complexes to identify a clone from one of the subtracted libraries which is
11 differentially expressed above a threshold level with respect to the subtracted libraries.

1 30. The method of claim 23, wherein the cellular characteristic is cell
2 health, the test cell is a diseased cell and the control cell is a healthy cell, and the
3 candidate gene is potentially correlated with a disease.

1 31. The method of claim 30, wherein the test cell is obtained from a
2 mammal that has had a stroke or is at risk for stroke.

1 32. The method of claim 30, wherein the test cell is obtained from a
2 mammal that has a neurological disease or develop phenotypes mimicing human
3 neurological diseases.

1 33. The method of claim 23, wherein the cellular characteristic is stage
2 of development and the test cell and the control cell are at different stages of
3 development, and the candidate gene is potentially correlated with mediating the change
4 between the different stages of development.

1 34. The method of claim 23, wherein the cellular characteristic is
2 cellular differentiation and the candidate gene is potentially correlated with controlling
3 cellular differentiation.

1 35. The method of claim 23, wherein the candidate gene is an
2 endogenous gene of the reference cell.

1 36. The method of claim 23, wherein the candidate gene is present in
2 the reference cell as an extrachromosomal gene.

- 1 37. The method of claim 17, wherein the reference cell is part of a cell
2 culture.
- 1 38. The method of claim 17, wherein the reference cell is part of a
2 tissue.
- 1 39. The method of claim 17, wherein the reference cell is part of an
2 organism.
- 1 40. The method of claim 17, wherein the reference cell is part of an
2 embryo.
- 1 41. The method of claim 17, wherein the reference cell is a mammalian
2 cell.
- 1 42. The method of claim 17, wherein the reference cell is a neural or
2 glial cell.
- 1 43. The method of claim 42, wherein the reference cell is a
2 neuroblastoma cell.
- 1 44. The method of claim 43, wherein the reference cell is useful as a
2 model system for investigating neurological disease in humans.
- 1 45. The method of claim 44, wherein the reference cell has increased
2 sensitivity to N-methyl-D-aspartate, β -amyloid, peroxide, oxygen-glucose deprivation, or
3 combinations thereof.
- 1 46. The method of claim 45, wherein the detecting step comprises
2 detecting a decrease in cellular sensitivity to N-methyl-D-aspartate, β -amyloid, peroxide,
3 oxygen-glucose deprivation, or combinations thereof.
- 1 47. The method of claim 17, wherein the detecting step comprises
2 detecting modulation of ligand binding to a protein.
- 1 48. The method of claim 17, wherein the reference cell is a part of an
2 organism and the detecting step comprises detecting a change in phenotype.

1 49. The method of claim 17, wherein the determining step comprises
2 determining whether interference with expression of the candidate gene in the reference
3 cell is correlated with alteration of a cellular activity or cellular state.

1 50. The method of claim 49, wherein interference is achieved by
2 introducing a double-stranded RNA into the reference cell that can specifically hybridize
3 to the candidate gene.

1 51. The method of claim 17, wherein the determining step comprises
2 determining whether the protein encoded by the candidate gene binds to another protein
3 to form a complex that can be coimmunoprecipitated.